Notes on the Design of Bioequivalence Study: Levofloxacin

Notes on the design of bioequivalence studies with products invited to be submitted to the WHO Prequalification Team – Medicines (PQTm) are issued to aid manufacturers with the development of their product dossier. Deviations from the approach suggested below can be considered acceptable if justified by sound scientific evidence.


Below, additional specific guidance is provided on the invited immediate release products containing levofloxacin.

**Pharmacokinetics of levofloxacin**

Orally administered levofloxacin is rapidly and almost completely absorbed with peak plasma concentrations being obtained within 1-2 h. The absolute bioavailability is 99-100%. Food has little effect on the absorption of levofloxacin. The tablets may be taken during meals or between meals. Levofloxacin is eliminated relatively slowly from the plasma (t½: 6 - 8 hours). Levofloxacin obeys linear pharmacokinetics over a range of 50 to 1000 mg.

**Guidance for the design of bioequivalence studies**

Taking into account the pharmacokinetic properties of levofloxacin the following guidance with regard to the study design should be taken into account:

**Design:** A cross-over design is recommended.

**Dose:** As the EoI includes levofloxacin 100 mg dispersible tablets and levofloxacin 250 mg tablet or capsule, 500 mg tablet, and 750 mg tablet, the bioequivalence study should be conducted preferably with the highest strength of the product series, e.g. 100 mg for the dispersible tablets and 750 mg for the tablets or 250 mg for the capsules, if all strengths are developed and the requirements for the additional strength biowaiver are fulfilled.

The bioequivalence study for the tablet could be waived according to the requirements for Biopharmaceutics Classification System (BCS) biowaivers since levofloxacin is classified as a BCS class I drug. However, for the capsule and the dispersible tablet, the BCS biowaiver is not possible since the comparator product is a tablet.

When conducting bioequivalence studies, it is essential to administer the test and the comparator product according to their corresponding instructions for use. In those cases where the test and the comparator product are different dosage forms with different methods of administration (e.g. a tablet that should be taken with a glass of water, and a dispersible tablet to be dispersed in 10 mL of water) the bioequivalence study should be conducted employing the intended method of administration of each product. It is considered incorrect to standardise the volume of liquid in all these cases (e.g. administering a glass of water after the intake of a dispersible tablet or rinsing the container where a dispersible tablet has been suspended with the remaining liquid of a glass of water) because this standardisation does not occur in real life conditions.
**Fasting/fed:** The bioequivalence study should be conducted in the fasted state.

**Subjects:** Healthy adult subjects should be utilized. It is not necessary to include patients in the bioequivalence study.

**Sample size:** Information currently available to PQTm indicates that the intra-subject variability for levofloxacin $C_{\text{max}}$ is around 15 - 18%. These data may facilitate the calculation of a sufficient sample size for a cross-over bioequivalence study.

**Washout:** Taking into account the elimination half-life of levofloxacin in healthy volunteers of 8 hours, a washout period of seven days is considered sufficient to prevent carry over.

**Blood sampling:** The blood sampling should be intensive for the first hours after administration to properly characterize the $C_{\text{max}}$ of levofloxacin. Sampling times beyond 24 are not necessary for the quantification of levofloxacin. For example, blood samples might be taken at predose, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50, 2.75, 3.00, 3.50, 4.00, 6.00, 8.00, 12.00, 16.00 and 24.00 h after drug administration.

**Analytical considerations:** Information currently available indicates that it is possible to measure levofloxacin in human plasma using LC-MS/MS analytical methodology. The bioanalytical method should be sufficiently sensitive to detect concentrations that are 5% of the $C_{\text{max}}$ in most profiles of each formulation (test or comparator).

**Parent or metabolite data for assessment of bioequivalence:** The parent drug is considered to best reflect the biopharmaceutical quality of the product. The disposition of levofloxacin should be characterized and the determination of bioequivalence will be based on the parent compound.

**Statistical considerations:** The data for levofloxacin should meet the following bioequivalence standards in a single-dose, crossover design study:

- The 90% confidence interval of the relative mean $AUC_{0-t}$ of the test to reference product should be within 80–125%
- The 90% confidence interval of the relative mean $C_{\text{max}}$ of the test to reference product should be within 80–125%.